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Supplemental Material to:

Kaori Sakuishi, Shin Foong Ngiow, Jenna Sullivan, Michele Teng, Vijay Kuchroo, Mark J. Smyth and Ana C. Anderson

Tim-3+FoxP3+ regulatory T cells are tissue specific promoters of T cell dysfunction in cancer

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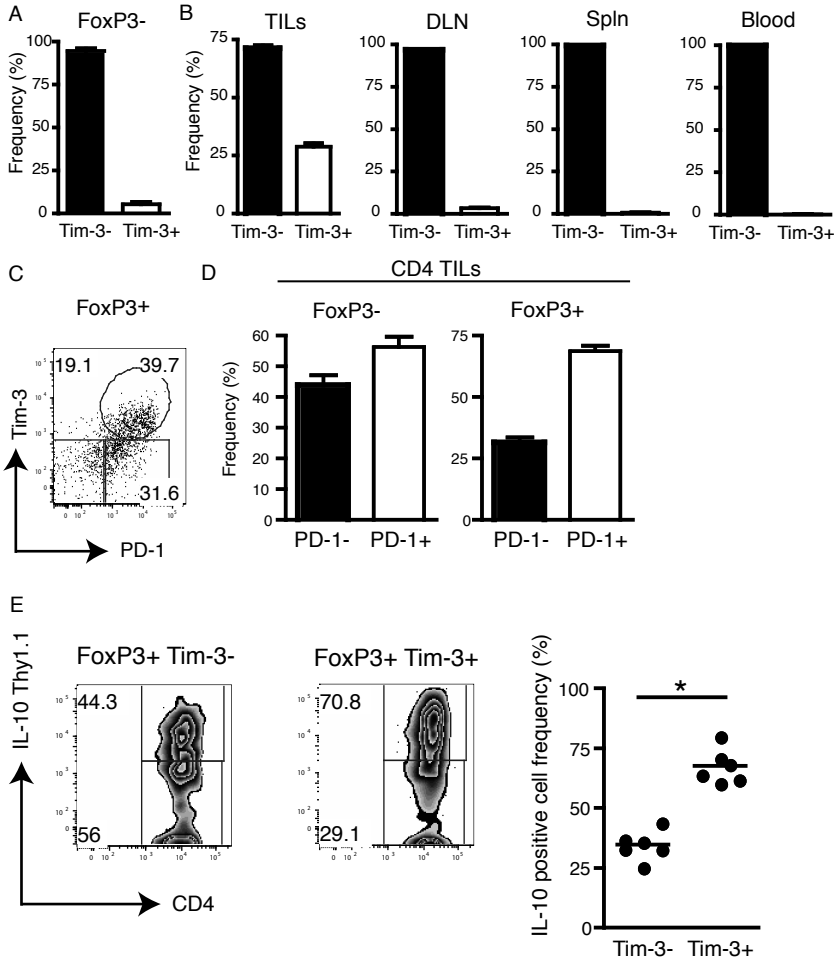
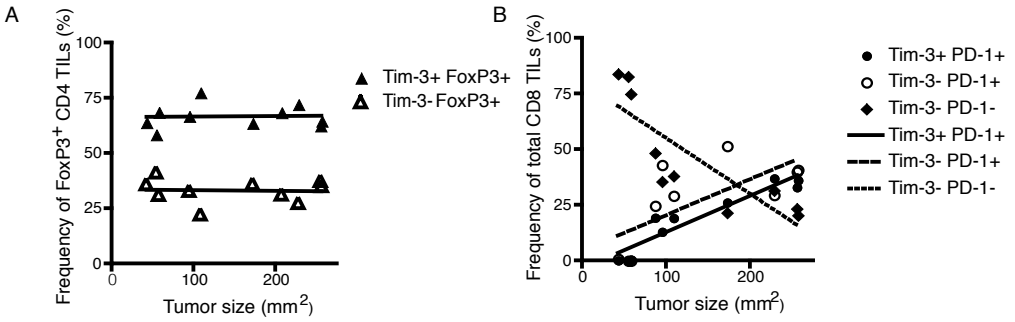
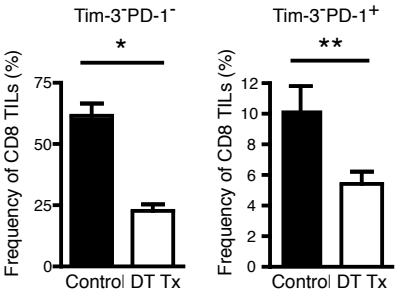
Supplementary Figure 1.



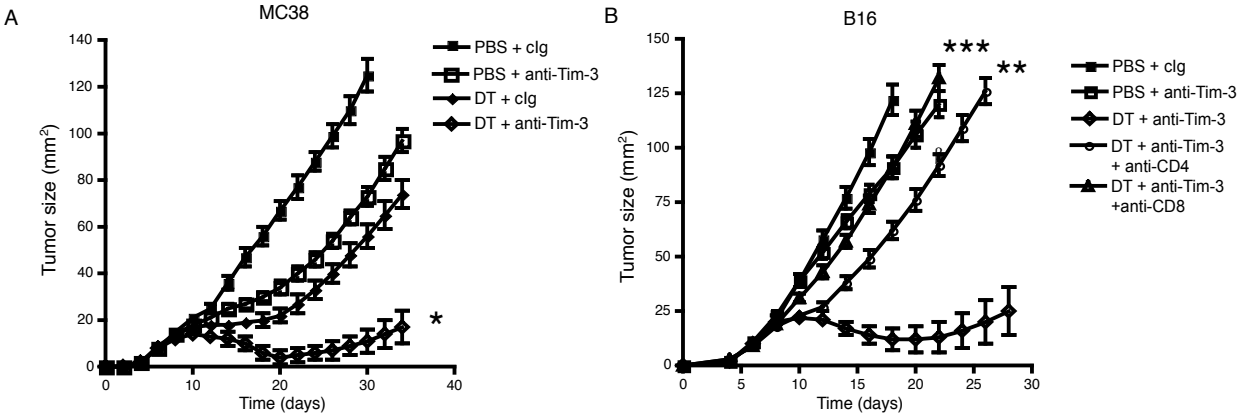
Supplementary Figure 2.



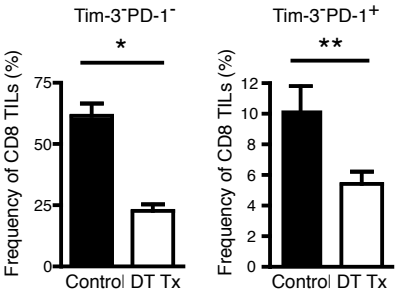
Supplementary Figure 3.



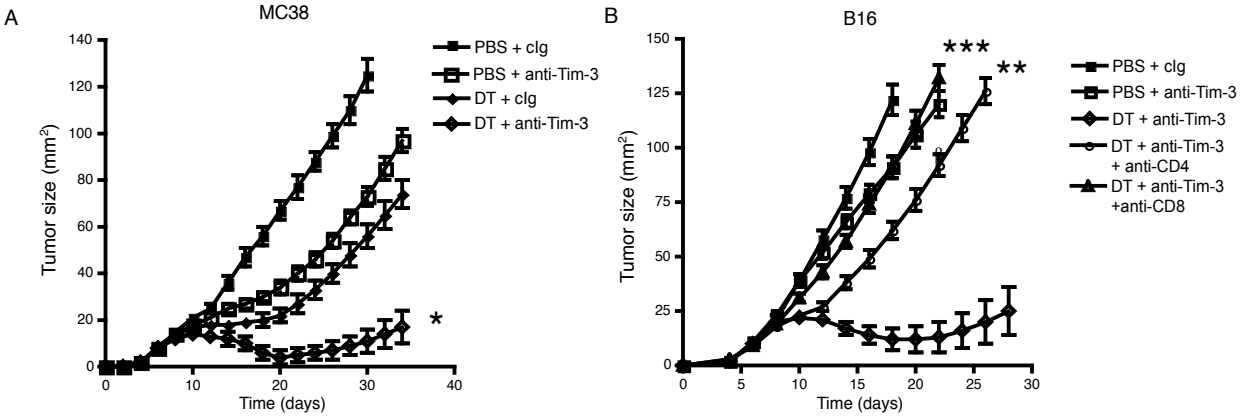
Supplementary Figure 4.



Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure Legends

Fig S1. Characterization of Tim-3$^+$ Treg in cancer. (A) Frequency of Tim-3$^-$ and Tim-3$^+$ cells in FoxP3$^-$ CD4$^+$ TILs in CT26. (B) Frequency of Tim-3$^-$ and Tim-3$^+$ cells in FoxP3$^+$ CD4$^+$ T cells in TILs, draining LN, spleen and blood from MC38 tumor-bearing mice. Error bars indicate standard error of the mean (SEM). (C) Representative flow cytometry showing co-expression of Tim-3 and PD-1 on FoxP3$^+$ CD4$^+$ TILs from CT26 colon carcinoma. (D) Frequency of PD-1$^+$ and PD-1$^-$ in FoxP3$^-$ and FoxP3$^+$ CD4$^+$ TILs. Error bars indicate standard error of the mean (SEM). (E) Left panel, representative intracytoplasmic staining for IL-10 in Tim-3$^+$ vs Tim-3$^-$ FoxP3$^+$ CD4$^+$ TILs from B16 tumor. Right panel, frequency of IL-10 positive cells in Tim-3$^+$ vs Tim-3$^-$ FoxP3$^+$ CD4$^+$ TILs from B16 (n=6). *t-test, $p<0.0001$.

Fig S2. Kinetic study of Tim-3 expressing CD4 and CD8 TILs subpopulation in CT26 tumor. (A) Frequency of Tim-3$^+$ and Tim-3$^-$ cells in FoxP3$^+$ CD4$^+$ TILs. (B) Frequency of CD8$^+$ TILs expressing Tim-3 and PD-1. Slopes were drawn by linear regression analysis.

Fig S3. Alteration in $CD8^+$ TILs populations following $FoxP3^+$ T cell depletion. Frequency of $Tim\text{-}3^-PD\text{-}1^-$ and $Tim\text{-}3^-PD\text{-}1^+$ cells in control versus DT treated mice. t-test, *p<0.0001, **p=0.0105. Error bars indicate standard error of the mean (SEM).

Fig. S4. Synergistic effect of Tim-3/Tim-3L pathway blockade and FoxP3 regulatory T cell depletion in MC38 and B16. DEREG mice were implanted with 1 x 10^6 MC38 (A) or 1 x 10^5 B16 (B) tumor and treated with either 1 μg DT (days 6, 8, 15 and 22) or PBS and 250 μg control Ig or anti-Tim-3 (days 8, 12, 16 and 20). In addition, groups of mice with B16 tumor received either anti-CD4 or anti-CD8 antibody as described in Figure 6. t-test conducted between the groups as indicated. *DT/cIg vs DT/anti-Tim-3 treated group in MC38 model, p=0.0278 day 15, p=0.02 day 20, p=0.0119 day 22-34; ** DT/ anti-Tim-3 vs DT/anti-Tim-3 with anti-CD4 depletion in B16, p=0.019 day 14, p=0.0079 day 16-22; *** DT/anti-Tim-3 vs DT/anti-Tim-3 with anti-CD8 depletion in B16, p=0.0117 day 12, p=0.0079 day 14-22.